



PATENT  
Atty. Docket No.: A-68970-1/RMS/DCF [469249-00154]

*In re* application of: ) Examiner: CHAKRABARTI, Arun K.  
FAN *et al.* )  
Serial No.: 09/785,514 ) Group Art Unit: 1634  
Filed: February 16, 2001 )  
For: PARALLEL GENOTYPING OF )  
MULTIPLE PATIENT )  
SAMPLES )

CERTIFICATE OF MAILING

I hereby certify that this correspondence, including listed enclosures, is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Commissioner for Patents, BOX RCE, Washington, DC 20231

Dated: February 14, 2003

Signed: Mari Kleineidam  
Mari Kleineidam

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DECLARATION PURSUANT TO 37 C.F.R. §1.131 TECH CENTER 1600/2900

Commissioner for Patents  
Washington, DC 20231

Sir:

We, Mark S. CHEE and Jian-Bing FAN hereby declare as follows:

1. We are the inventors on the above-identified patent application and are familiar with its contents. We have also reviewed the pending claims in this application.
2. We are familiar with the Office Action mailed on August 14, 2002 wherein claims 14-29 were rejected over Chee et al. (WO 99/67641) published December 29, 1999.
3. All of the ideas detailed in the above-identified application were contemplated in this country prior to December 29, 1999. This is evidenced by the appended documents.
4. Appended DOCUMENT 1 comprises pages 1-6 of an Illumina Invention Disclosure Form (with dates redacted). A summary of the invention is presented in the section numbered "4. Describe the invention" and in the attached

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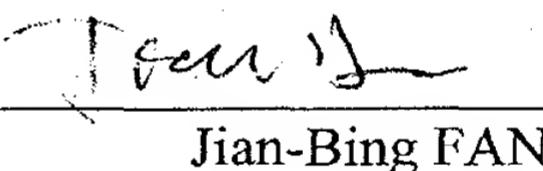
experimental scheme. This Invention Disclosure Form was signed and witnessed prior to December 29, 1999.

5. In conclusion, the invention was completed in this country prior to December 29, 1999.
6. We declare further that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that the making of willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issuing thereon.

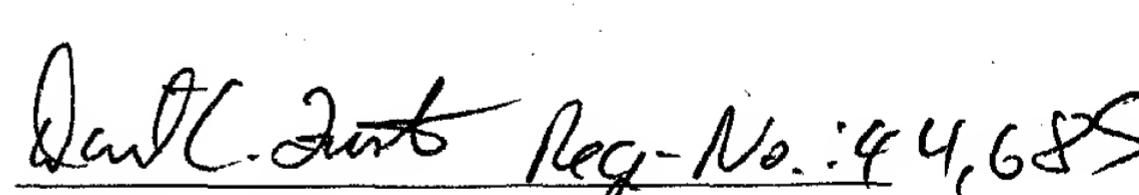
Date: 1/20/03

  
Mark S. CHEE

Date: 1/16/2003

  
Jian-Bing FAN

Dated: 2/14/2003

  
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## INVENTION DISCLOSURE FORM

This form is provided to permit evaluation of the patent potential of company inventions, and to facilitate preparation of patent applications when warranted. Please fill in each space as completely as possible, and use additional sheets when necessary.

1. Name: Jian-Bing Fan and Mark Chee

2. Date:

3. State the Title of the Invention:

**Parallel genotyping of SNPs in multiple individuals by direct sample immobilization and allele-specific oligonucleotide (ASO) hybridization.**

4. Describe the invention: Use additional sheets if necessary. Attach descriptive materials such as drawings, sketches, photographs, etc. which may help illustrate the invention. Delineate new and important features. Make sure to include both the preferred embodiment as presently identified, and alternative constructions, procedures or equivalent components which can accomplish the same result as the preferred embodiment.

**We describe here an effective approach for parallel genotyping in many individuals (please see attached experimental scheme).**

**This approach can be used not only to genotype SNPs in individual samples, but also to estimate allele frequencies in pooled samples. Furthermore, this method can be used in cytogenetic mapping and physical mapping of genetic markers and DNA clones.**

5. State the primary purpose of the invention, including the need satisfied or problem solved by the invention:

**The invention provides a straightforward and cost-effective approach for high-throughput genotyping with many samples.**

6. Please list what you feel is the prior art: please include references, articles, talks, abstracts, patents, etc. which are relevant to either the state of the prior art or to the invention. Please include dates and provide copies whenever possible:

References on dot-blot hybridization for genotyping (attached):

- 1) Shuber et al., (1997) Human Molecular Genetics 6, 337-347.
- 2) Nozari et al. (1988) Analytical Chemistry 172, 180-184.
- 3) Conner et al. (1983) PNAS 80, 278-282.
- 4) Wallace et al. (1979) Nucleic Acids Research 6, 3543-3557.

We are not aware of prior art on this topic. The novelty of the approach described here lies in the combination of randomly assembly of many different types of beads (representing samples from many different individuals) on a single fiber bundle and the sequential parallel ASO hybridization and beads decoding. In this way, sample to sample variation can be normalized on the same fiber bundle.

Given the hybridization efficiency and the power of the Array of Arrays™ approach that Illumina is taking, this method provides an efficient approach for genotyping and other mapping applications (see above).

7. Are there any publications, abstracts, submitted manuscripts, talks, etc. on this work (either already done or in the works)? Please provide details and dates:

**None.**

8. Compare new and important features of the invention with the prior art, explaining why and how the invention is better:

The experimental scheme proposed here combines the fast sequential parallel ASO hybridization and beads decoding, which enables genotyping multiple samples in parallel on a single fiber bundle. Thereafter, genotyping throughput is increased. In addition, the method described is very straightforward, thus easier to implement and can be cost-effective.

9. Please list known competitors or alternate technologies which solve the same problem:

10. Are there commercial products you envision? Please describe:

11. What are the immediate research plans or steps to be taken:

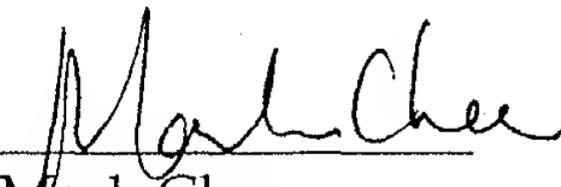
12. What are the longer term research plans or steps to be taken:

13. Earliest date and place invention was conceived, and substance of conception (identify people and records to support date and place, such as notebook numbers and pages):

14. Name, title, signature, and address of each person who made an intellectual contribution to the invention described in this disclosure:



Jian-Bing Fan  
Senior Scientist, Illumina  
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Mark Chee  
Vice President, Illumina  
155 15<sup>th</sup> Street #24  
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15. Name and signature of two witnesses who are not inventors who understand the technical aspects of this invention:

AWG  
Anthony W. Gzarnik

SB  
Steven M. Bernard



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1. Sample preparation:

Specific genomic regions (e.g. regions containing SNPs) from different individuals are PCR-amplified or enriched by other methods.

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- The PCR-amplification can be multiplexed. For example, multiplexing PCR can be used to amplify 100s to 1000s SNPs from each individual.
- The PCR amplification can be achieved with genomic region-specific primers, or generic primers, for example, Alu-primers and degenerated primers.
- The amplified genomic DNAs can be labeled with chemically modified dNTPs during PCR or after PCR.

2. Immobilization:

Specifically genomic regions amplified from different individuals (see above) will be separately immobilized onto differentially coded beads. (The beads can be coded in a variety of ways. For example, each set of nucleic acids immobilized onto a bead population can contain a reference nucleic acid which is used as an identifier for the bead population).

- For example, 100 bead types can be used in immobilization of DNA samples from 100 individuals.
- Immobilization can be achieved through many different approaches. For example, Biotin-streptavidin conjugation, Phenylboronic Acid (PBA) and Salicylhydroxamic Acid (SHA) conjugation, covalent immobilization through amide formation, or cross-linking.

3. Mixing and assembly of beads on BeadArrays™:

Beads corresponding to samples from different individuals will be pooled together and loaded onto fiber bundles.

- For example, 100 bead types representing samples from 100 individuals can be pooled together. Thus, if 1,000 SNPs are amplified from each individual, 100,000 (1000 SNPs/individual x 100 individuals = 100,000 genotypes) genotypes can be generated from one BeadArray™.
- Alternatively, each bead type on an array can contain a different subset of SNPs from the same individual. Thus, based on the above example, 100,000 genotypes can be obtained from a single individual using a

single BeadArray™ which contains 100 subpopulations of beads. BeadArrays™ with larger numbers of subpopulations will contain correspondingly larger amounts of information.

#### **4. Sequential ASO hybridization and decoding:**

For each SNP, oligonucleotides corresponding to the two specific alleles will be differentially color-labeled and hybridized to the fiber bundles.

- Many SNP-specific ASOs will be hybridized to the fiber bundle sequentially.
- Multiple oligonucleotides corresponding to the two specific alleles of several different SNPs can be differentially color-labeled and hybridized to the fiber bundles simultaneously.
- Beads on the fiber bundles can be decoded as described in previous Illumina's patents/protocols.

#### **5. Data analysis:**

Hybridization patterns with SNP-specific ASOs will be computed and used to call genotypes in each samples.

- Clustering analysis will be performed and sample to sample variation will be normalized on the same fiber bundle.